Chapter 31
Proposal of International Gluten Research Group

Tatsuya M. Ikeda, W. John Rogers, Gerard Branlard, Roberto J. Peña, Silvia E. Lerner, Adriana Arrigoni, Wujun Ma, Rudi Appels, Odean Lukow, William Hurkman, Marie Appelbee, Mike Sissons, Jose M. Carrillo, and Zhonghu He

Abstract In a scenario of climate change and rapidly rising urban populations demanding processed foods, it is necessary to develop new wheat cultivars combining high yield potential, disease resistance, and stability for yield and processing quality, even under heat or drought stress conditions. Allelic variation for gluten proteins (glutenin subunits and gliadins) is a major determinant of differences in dough viscoelastic properties observed between cultivars of both bread wheat and durum wheat. Technical difficulties in allelic identification due to the complexity of the protein profile produced by each cultivar and the use of different nomenclature systems in different laboratories has historically interfered with information exchange between research groups, a situation exacerbated by the vast number of possible profiles found in different cultivars due to the multi-allelic nature of the principal loci encoding gluten proteins (*Glu-1*, *Glu-2, Glu-3*, *Gli-1* and *Gli-2*). For the *Glu-3* alleles, we have collaborated to unify criteria across laboratories and to

T.M. Ikeda (✉)
NARO, Western Region Agricultural Research Center, Fukuyama, Japan
e-mail: tmikeda@affrc.go.jp

W.J. Rogers
CIISAS, CICPBA-BIOLAB AZUL, DCBAyB, Facultad de Agronomía, CONICET-INBIOTEC, UNCPBA, Azul, Argentina

G. Branlard
INRA, UMR1095 Genetics, Diversity and Ecophysiology of Cereals, 5 chemin de Beaulieu, Clermont-Ferrand F-63 039, France

Blaise Pascal University, UMR1095 Genetics, Diversity and Ecophysiology of Cereals, Aubière F-63173, France

R.J. Peña
CIMMYT, Texcoco, Mexico

S.E. Lerner • A. Arrigoni
CRESCAA, Facultad de Agronomía, UNCPBA, Azul, Argentina

W. Ma • R. Appels
Western Australia Department of Agriculture and Food, State Agriculture Biotechnology Center, Murdoch University, Murdoch, Australia

Y. Ogihara et al. (eds.), *Advances in Wheat Genetics: From Genome to Field*, DOI 10.1007/978-4-431-55675-6_31

compare four different methods of allelic identification (SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR), and have shown that the four methods can be regarded as complementary techniques for allelic identification. We seek to continue addressing remaining analytical challenges, place the findings in the context of the Catalogue of Gene Symbols for Wheat, and, with unified criteria, initiate work to define better the relationship between specific gluten proteins and processing quality attributes. Therefore, we propose a new system to share materials through public gene banks in collaboration with the Catalogue, and the formation of a wider international group aimed at facilitating the resolution of the remaining problems in the field. We also propose to extend our collaboration by forming a wheat quality expert working group under the Wheat Initiative.

Current Status of *Glu-3* Allele Nomenclature

It has been shown that allelic variation for the high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs) affects the properties of dough made with different wheat cultivars. LMW-GS composition in common wheat is one of the critical determinants of gluten properties (Branlard et al. 2001; Eagles et al. 2006; Gupta et al. 1994; Liu et al. 2005; Maruyama-Funatsuki et al. 2005). Gupta and Shepherd (1990) assigned the individual LMW-GSs to *Glu-A3*, *Glu-B3* and *Glu-D3* loci and selected standard cultivars that covered the allelic variation observed. However, subsequent use of *Glu-3* nomenclature has not been consistent among laboratories, due to the complexity of the LMW-GSs, different separation methods and different standard cultivars used by researchers (Branlard et al. 2003; Ikeda et al. 2006; Singh et al. 1990). It is

O. Lukow
Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, Canada

W. Hurkman
USDA Agricultural Research Service, Western Regional Research Center, Albany, CA, USA

M. Appelbee
South Australian Research and Development Institute, Adelaide and LongReach Plant Breeders, Lonsdale, Australia

M. Sissons
Tamworth Agricultural Institute, Calala, NSW, Australia

J.M. Carrillo
Unidad de Genética, ETSIA, Madrid, Spain

Z. He
Institute of Crop Science, National Wheat Improvement Center/The National Key Facility for Crop Genetic Resources and Genetic Improvement, CAAS, Beijing and CIMMYT, China Office, Beijing, China

necessary to unify the various *Glu-3* allelic nomenclature systems in use, to allow information to be shared regarding the effects of individual alleles on gluten properties and to be applied in breeding programs aimed at improving gluten properties. In previous studies, four laboratories plus an international institution shared cultivars and compared results. We confirmed that there were inconsistencies to identify *Glu-3* alleles between laboratories due to differences of separation and identification methods (Ikeda et al. 2008). Using 2-DE analysis, we found new *Glu-3* alleles among these materials (Ikeda et al. 2009). Combining SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR analyses, we showed that a combination of methods was required to identify certain alleles, and that these methods would be especially useful when characterizing new alleles. We also recommended 30 cultivars as standards for the determination of LMW-GS alleles (Liu et al. 2010).

Sharing Materials

It is very important to share materials to unify nomenclature among research groups. However, it is not always possible to obtain cultivars representing *Glu-3* alleles listed in Catalogue of Gene Symbols for Wheat (McIntosh et al. 2013). Therefore, we need to establish a system to share materials internationally. We propose to deposit cultivars representing particular alleles in public gene banks (e.g. Germplasm bank in CIMMYT, Genebank in VIR in Russia, NBRP in Japan, and GRIN in the USA). The registered alleles will be available publicly through these gene banks. New alleles can be evaluated by curators of the Catalogue and other researchers for registration in the catalogue. This system also helps to refine the catalogue (Fig. 31.1). At present CIMMYT Genebank performs seed multiplication of a *Glu-3* common wheat master set.

Sharing Methods

It is also important to use common methods to identify *Glu-3* alleles. For SDS-PAGE, Peña proposed the use of separation gels containing Tris buffer of pH 8.5 instead of pH 8.8 for better separation of LMW-GS bands (Ikeda et al. 2008; Peña et al. 2004). Lowering bis-acrylamide concentration and using larger size gels also helps better separation (Branlard et al. 2003). Further evaluation for creating a standard SDS-PAGE method is necessary. For PCR markers, as the number of known alleles increases, we need to reconfirm the usefulness of PCR markers to identify *Glu-3* alleles. For example, a PCR primer set, which was developed to identify the *Glu-B3i* allele (Wang et al. 2009), identified the *Glu-B3ad* allele instead. It is necessary to select a standard PCR primer set to identify *Glu-3* alleles.

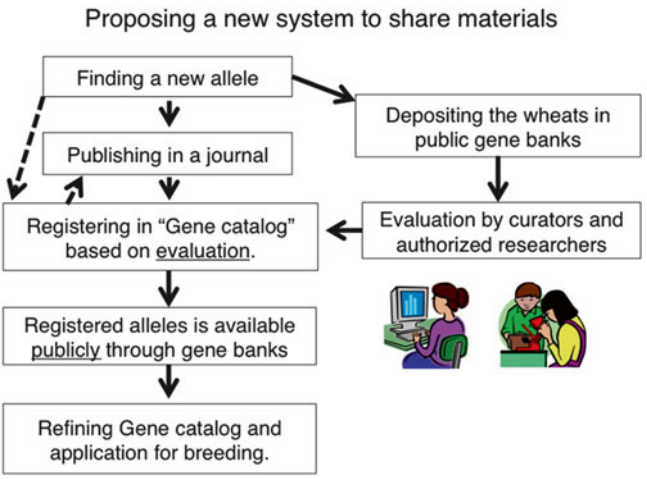

Fig. 31.1 A new system to share materials for gluten analysis

Functional Analysis of Gluten Proteins

By sharing materials and methods among international research groups, it becomes possible to define better the relationship between specific gluten proteins and processing quality stability, even under heat or drought stress wheat growing conditions. We will set an international framework to evaluate allelic effects on quality attributes under various environmental conditions.

Unification with Durum *Glu-3* Alleles

Durum *Glu-3* alleles were classified independently of those of common wheat (Martinez et al. 2004; Nieto-Taladriz et al. 1997). In the Catalogue, the durum *Glu-3* alleles were originally assigned separately and subsequently combined into one provisional list. Since tetraploid durum wheat shares common ancestral species with common wheat, we would expect some alleles to be identical to those of common wheat. We shared standard cultivars and studied *Glu-3* alleles by SDS-PAGE, 2-DE and PCR. Some alleles seemed to share the same alleles with common wheat, but some were unique in durum wheat (data not shown). This means that durum allele might widen the genetic diversity of common wheat alleles, and vice versa. Further analysis is necessary to clarify durum *Glu-3* alleles and produce a definitive list in the Catalogue for use by the wheat community. This is also important for *Glu-1* alleles.

Gliadin Analysis

Gliadin consists of α/β/γ/ω-gliadins, which contain many proteins having a range of molecular weights and pI values. Variation in the gliadins also effects dough properties (Branlard and Dardevet 1994). Gliadins are also known to contain epitopes involved in wheat gluten related disorders (Sapone et al. 2012). Gliadin analysis was mainly carried out using A-PAGE. The analysis of gliadin proteins using SDS-PAGE allows the determination of the banding patterns associated with the close linkage existing between *Gli-1* and *Glu-3*, and, therefore, this approach further contributes to the identification of specific *Glu-3* LMW-GS in both common and durum wheat. With increasing genome sequence data availability, it is important to identify gliadins by proteomic techniques to clarify correspondence between gliadin proteins, the epitopes of allergen and coding genes (Juhasz et al. 2012).

Forming an International Gluten Research Group

To carry out these tasks, we propose to form an international gluten research group. Using the same materials under different environmental conditions makes it possible to evaluate the effects of *Glu-3* alleles on dough properties in such conditions. From this collaboration, we can share advanced knowledge of gluten function for further study, accelerating the development of new cultivars maintaining good quality under climate change and responding to quality demands from industries and consumers (Fig. 31.2). It is also possible to use gluten protein alleles for cultivar identification to protect breeder's rights.

Further Perspective

The field of gluten research overlaps other wheat quality related fields, e.g. allergy, nutrition and carbohydrates. Therefore, it is logical to attempt extending our collaboration to other researchers related to wheat quality. Currently we work to form a wheat quality expert working group under the Wheat Initiative (http://www.wheatinitiative.org/about/expert-working-groups). We would like to invite other colleagues related to wheat quality to join our collaboration.

Fig. 31.2 Objectives and targets for forming an international gluten research group

References

Branlard G, Dardevet M (1994) A null *Gli-D1* allele with a positive effect on bread wheat quality. J Cereal Sci 20:235–244

Branlard G, Dardevet M, Saccomano R et al (2001) Genetic diversity of wheat storage proteins and bread wheat quality. Euphytica 119:59–67

Branlard G, Dardevet M, Amiour N, Igrejas G (2003) Allelic diversity of HMW and LMW glutenin subunits and omega-gliadins in French bread wheat (*Triticum aestivum* L.). Genet Res Crop Evol 50:669–679

Eagles HA, Cane K, Eastwood RF et al (2006) Contributions of glutenin and puroindoline genes to grain quality traits in southern Australian wheat breeding programs. Aust J Agric Res 57:179–186

Gupta RB, Shepherd KW (1990) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutelin. 1. Variation and genetic control of the subunits in hexaploid wheats. Theor Appl Genet 80:65–74

Gupta RB, Paul JG, Cornish GB et al (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1*, of common wheat. I. Its additive and interaction effects on dough properties. J Cereal Sci 19:9–17

Ikeda TM, Araki E, Fujita Y, Yano H (2006) Characterization of low-molecular-weight glutenin subunit genes and their protein products in common wheats. Theor Appl Genet 112:327–334

Ikeda TM, Branlard G, Peña RJ, et al (2008) International collaboration for unifying *Glu-3* nomenclature system in common wheats. In: Appels R, Eastwood R, Lagudah E, et al (eds) The 11th international wheat genetics symposium, Sydney University Press, Brisbane

Ikeda TM, Branlard G, Peña RJ, et al (2009) Characterization of new *Glu-3* alleles in bread wheat. In: INRA (ed) 10th international gluten workshop, INRA, Clermont-Ferrand, pp 180–184

Juhasz A, Gell G, Bekes F, Balazs E (2012) The epitopes in wheat proteins for defining toxic units relevant to human health. Funct Integr Genomics 12:585–598

Liu L, He Z, Yan J et al (2005) Allelic variation at the *Glu-1* and *Glu-3* loci, presence of the 1B.1R translocation, and their effects on mixographic properties in Chinese bread wheats. Euphytica 142:197–204

Liu L, Ikeda TM, Branlard G et al (2010) Comparison of low molecular weight glutenin subunits identified by SDS-PAGE, 2-DE. MALDI-TOF-MS and PCR in common wheat. BMC Plant Biol 10:124

Martinez MC, Ruiz M, Carrillo JM (2004) New B low Mr glutenin subunit alleles at the *Glu-A3*, *Glu-B2* and *Glu-B3* loci and their relationship with gluten strength in durum wheat. J Cereal Sci 40:101–107

Maruyama-Funatsuki W, Takata K, Funatsuki H et al (2005) Identification and characterization of a novel LMW-s glutenin gene of a Canadian Western extra-strong wheat. J Cereal Sci 41:47–57

McIntosh RA, Yamazaki Y, Dubcovsky J, et al (2013) Catalogue of gene symbols for wheat. In: 12th international wheat genetics symposium, Yokohama

Nieto-Taladriz MT, Ruiz M, Martínez MC et al (1997) Variation and classification of B low-molecular-weight glutenin subunit alleles in durum wheat. Theor Appl Genet 95:1155–1160

Peña RJ, Gonzalez-Santoyo H, Cervantes F (2004) Relationship between some *Glu-D1*/*Glu-B3* allelic combinations and bread-making quality related parameters commonly used in wheat breeding. In: Lafiandra D, Masci S, D'Ovidio R (eds) The gluten proteins. Royal Society of Chemistry, Cambridge, pp 156–157b

Sapone A, Bai JC, Ciacci C et al (2012) Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med 10:13

Singh NK, Shepherd KW, Cornish GB (1990) A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J Cereal Sci 14:203–208

Wang L, Zhao X, He Z et al (2009) Characterization of low-molecular-weight glutenin subunit *Glu-B3* genes and development of STS markers in common wheat (*Triticum aestivum* L.). Theor Appl Genet 118:525–539